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Effectiveness of stimulating PCE reductive dechlorination: A step-wise approach

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ABSTRACT

Reductive dechlorination of tetrachloroethene (PCE) and its daughter products in aquifers is often hampered by Fe(III) reducing conditions. Rigorous treatment to adjust the redox potential and stimulate dechlorination may be costly and potentially have negative effects on other aquifer functions. A step-wise experimental strategy was applied to investigate the effectiveness of various adjustment scenarios. Batch experiments with ascorbic acid (AA) and sodium lactate (SL) showed that 75 μmol electron equivalents per gram dry mass of aquifer material was required to reach a sufficiently low redox potential for the onset of PCE dechlorination. Similar effects of either AA or SL on the measured redox potential suggest electron donors are not specific. However, the relative rates of Fe(III) and sulphate reduction appeared to be specific to the electron donor applied. While redox potential stabilised around −450 mV after titration and sulphate was reduced to zero in both treatments, in the AA treatment a faster production of Fe^{2+} was observed with a final concentration of 0.46 mM compared to only 0.07 mM in the SL treatment. In subsequent batch experiments with aquifer material that was pre-treated with AA or SL, PCE reductive dechlorination occurred within 30 days. Further stimulation tests with extra electron donor or inoculum revealed that adding electron donor can accelerate the initiation of PCE biodegradation. However, bioaugmentation with dechlorinating bacteria is required to achieve complete reductive dechlorination to ethene. The findings from step-wise approaches are relevant for improving the cost-effectiveness of the design and operation of in-situ bioremediation at initially unfavourable environmental conditions.

1. Introduction

Tetrachloroethene (PCE) was introduced as a dry cleaning solvent in the 1930s (ITRC, 2005; Longstaff et al., 1992). PCE together with its daughter products, trichloroethene (TCE), *cis*-dichloroethene (*cis*-DCE) and vinyl chloride (VC), which are commonly called volatile chlorinated ethenes (VOCls), are widespread groundwater contaminants throughout the world (Friis et al., 2007; Grindstaff, 1998; Henry Susan et al., 2002; WHO, 2004). Currently over 10,000 sites are contaminated with PCE in the Netherlands (Nipshagen and Praamstra, 2010). Meanwhile it was estimated that still more than 80% of the commercial dry cleaners use PCE in the United States in 2004 (Linn et al., 2004).

PCE has higher density than water; hence, not only can it spread through mobilisation of groundwater, but also sink down until trapped above finer grained layers. This can result in a large area of pure product contamination that is difficult to remediate with conventional techniques such as pump and treat (Grossett, 2001; ITRC, 2005; Luciano et al., 2010). PCE is potentially carcinogenic (Umezu et al., 1997; USEPA, 2012) and difficult to characterise and to remediate. Therefore many studies have been directed at microbiological ways to diminish the threat (Kästner, 1991a, 1991b; Kuchovsky and Sracek, 2007; Mulligan and Yong, 2004). Laboratory experiments

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(Freedman and Gossett, 1989; Knauss et al., 2000; Middeldorp et al., 1999; Prakash and Gupta, 2000; Ye et al., 2008) focused on elaborating the dechlorination pathway and bacteria involved in PCE biodegradation at optimal conditions, while pilot or field tests (Aulenta et al., 2007; Clement et al., 2000; Grindstaff, 1998; Major et al., 1991; Morrill et al., 2009) focused on stimulating bioremediation in groundwater or aquifer system by injecting sufficient substrate and bacteria.

PCE can be completely reduced to ethene via reductive dechlorination under sulphate reducing and methanogenic conditions (Holliger, 1992; McCarty, 1997) when other conditions like temperature, availability of electron donor and nutrients, and presence of specific microorganisms are suitable (Atteia and Guillot, 2007; Ballapragada et al., 1997; Bennett et al., 2007; Boopathy, 2000; Fowler and Reinauer, 2013; Holliger et al., 1993; Kästner, 1991a, 1991b; Tsui et al., 2011). Among these conditions, the redox condition proved to greatly affect the presence and activity of dechlorinating bacteria (Abe et al., 2009; ITRC, 2005; Lu et al., 2006; Takeuchi et al., 2011; van der Zaan et al., 2010), especially of "*Dehalococcoides*," which is the only group of bacteria capable to fully degrade VOCls to ethene. Reductive dechlorination of VOCls in aquifers is often hampered by unsuitable Fe(III) reducing conditions. Especially Fe(III) and SO_4^{2-} have been reported as main competitive electron acceptor to VOCls (Kouznetsova et al., 2010; McCarty, 1997; Zaa et al., 2010). Therefore, determining the redox condition is important in evaluating the reductive dechlorination potential of VOCls in the field. Redox potential as measured electrochemically can be used as an indicator for aquifer redox conditions. Limited research was done so far on the role of redox potential in reductive dechlorination processes of some chlorinated hydrocarbons, such as hexachloro-1,3-butadiene (HCBD) (Cord-Ruwisch et al., 2009), halogenated methanes (Olivas et al., 2002) and Pentachlorophenol (PCP) (Stuart et al., 1999). These studies showed that dechlorination can be monitored via the redox potential which can be used as an indicator of dechlorinating performance. However, research focused on assessing the direct relationship between redox potential and PCE reductive dechlorination is to our knowledge absent.

The aim of this study was to closely combine redox potential and PCE reductive dechlorination. Using redox potential as a criterion and indicator for PCE reductive dechlorination, we lowered the redox condition of aquifer material from Utrecht, Netherlands, by pre-treating the material with two different electron donors, ascorbic acid (AA) and sodium lactate (SL), in order to overcome the existing barrier for PCE biodegradation without adding excess electron donor, because excess electron donor may cause bio-chemical clogging in for example groundwater extraction wells or transport pipelines. Considering these possible negative interferences with other aquifer utilizations, and the ultimate view of application in large volumes of contaminated aquifer, we applied a step-wise approach, to estimate the minimum amount of electron donor needed for improving the redox potential of aquifer to initiate PCE reductive dechlorination.

2. Materials and methods

In summary, experiments consisted of two parts: (1) redox titrations with electron donor to estimate the minimum amount of electron donor needed to achieve redox potential values theoretically suitable for reductive dechlorination of PCE to occur; (2) PCE reductive dechlorination experiments in different combinations of pre-treatment and subsequent stimulation scenarios (Fig. 1). More detailed information and codes on the experiment part 2 are given in Table 1.

2.1. Basic materials

AA and SL were selected as electron donor, as AA has been widely used as a reactive chemical reductant for Iron(III) in the subsurface (Ambikadevi and Lalithambika, 2000; Chiarizia and Horwitz, 1991; Hyacinthe et al., 2006) and lactate as a well known biological reductant, has also been used in many studies (Call and Logan, 2011; Finke et al., 2007; Williamson et al., 2013). The half reactions of complete oxidation from AA or lactic acid to CO_2, as well as reduction of Fe(III) to Fe(II) and reduction of SO_4^{2-} to HS^- are given below:

$$C_6H_8O_6 + 6H_2O \rightarrow 6CO_2 + 20H^+ + 20e^- \quad (1)$$

$$C_3H_6O_3 + 3H_2O \rightarrow 3CO_2 + 12H^+ + 12e^- \quad (2)$$

$$Fe(III) + e^- \rightarrow Fe(II) \quad (3)$$

$$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O \quad (4)$$

Aquifer material was collected at a site near Utrecht Central Station, the Netherlands. The aquifer mainly consists of fine sands. VOCls, especially VC and *cis*-DCE, were found in this aquifer that is currently under Fe(III) reducing conditions. Details on the geochemical properties of the aquifer can be found in the report of Dinkla et al. (2012). Samples of this aquifer were selected since extensive re-construction of the area is being performed and a large number of groundwater extraction or injection wells are being installed or planned, for groundwater monitoring activities and aquifer thermal energy storage. Besides, this area was a case study area involved in the projects Meer Met Bodemenergie[1] and CityChlor.[2] The aquifer samples used in the experiments reported here came from a depth of 35–38 m below surface, where mostly VC and *cis*-DCE were present and with redox potential ranging from −112 to −151 mV.

All chemical solutions were made with anaerobic deionised water, which was first boiled and then purged with pure N_2 during cooling down to ambient temperature (22 °C). Ascorbic acid powder (≥99% purity, BDH Prolabo®) and sodium lactate powder (≥99% purity, Aldrich®) were used to prepare stock solutions of 18.9 g/L AA and 28.1 g/L SL respectively, for the redox titration experiment. These concentrations were aimed to be near-equivalent in electrons from AA and SL, based on complete oxidation to CO_2 (Eqs. (1) and (2)). Additional AA and SL stocks (200 g/L) were prepared for pre-treatments. PCE (99% anhydrous, Aldrich®) was used to prepare a stock

[1] http://www.meermetbodemenergie.nl.
[2] http://www.citychlor.eu/.

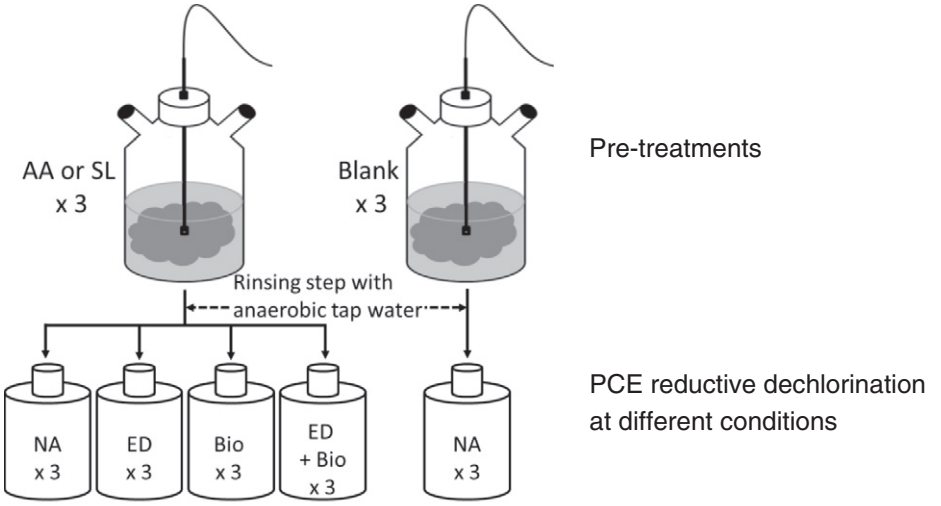

Fig. 1. Schematic representation of experimental set-up for the reductive dechlorination batch tests after pre-treatment with AA or SL. NA: natural attenuation; ED: stimulation with electron donor; Bio: stimulation with inoculum.

solution of 105 mg/L. Anaerobic tap water was used as the bulk liquid in the titration and conditioning experiments, following the same procedure as with the deionised water. All biological reductive dechlorination batches received anaerobic medium containing per litre: 1.09 g Na_2HPO_4; 0.53 g KH_2PO_4; 1 g NH_4Cl; 48 mg $CaCl_2 \cdot 2H_2O$; 54 mg $MgSO_4 \cdot 7H_2O$; 1.2 mg $FeCl_2 \cdot 4H_2O$; 1.2 mg $CoCl_2 \cdot 6H_2O$; 0.3 mg $MnCl_2 \cdot 4H_2O$; 0.018 mg $CuCl_2 \cdot 2H_2O$; 0.03 mg $ZnCl_2$; 0.03 mg HBO_3; 0.054 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$; 0.06 mg $Na_2SeO_3 \cdot 5H_2O$; 0.03 mg $NiCl_2 \cdot 6H_2O$; 0.6 mg EDTA (tripex II); 0.216 ml 36% HCl; 0.3 mg resazurin (oxygen and pH indicator).

A mixed dechlorinating culture, provided by Bioclear BV, was fed with 20 mmol/L lactate and PCE. After 20 days of cultivation, when PCE was completely degraded to ethene, this mixed culture was used as inoculum.

2.2. Analytical methods

Dry weight of the aquifer material was determined by the difference of weight before and after 24 h in a 105 °C oven.

Redox potential of the experimental systems was monitored by a Consort multi-channel (C3060) metre and data logger with ProSense (QIS) standard Pt redox electrode with Ag/AgCl as reference electrode (−199 mV vs. standard hydrogen electrode (SHE)) in saturated KCl solution (Bard and Faulkner, 2001). During redox monitoring, a relatively high noise level in redox readings was observed, especially in the blanks, in both the redox titration experiment and the pre-treatments. Besides, it was observed that gas or liquid sampling momentarily interfered with the redox readings. In this paper, the measured redox potentials are therefore presented as smoothed values from the raw data, using the "smooth (Y, 500, 'loess')" function in MATLAB,[3] which uses weighted linear least squares and a 2nd degree polynomial model[4] to fit and smooth the raw data. An example of raw data and smoothed data is provided in the supplementary material (see Fig. S1 and S2).

Dissolved Fe^{2+} concentration was determined with Hach Lange cuvette tests (LCK-320 0.2–6.0 mg/L Fe^{2+}/Fe^{3+}) on a Xion 500 spectrophotometer; SO_4^{2-} concentration was determined on a Dionex ICS 2100 with IonPac AS19 column and a conductivity detector. Analyses of AA and lactate were performed on HPLC with organic acids column (Ion 300) and refractive index (RI) detector. Volatile fatty acids (VFAs) were determined on a Hewlett-Packard (HP) 5890 series GC with packed column (10% Flurad on Supel-coport) and flame ionisation detector (FID). pH was determined with Dosatest pH indicator strips from VWR Prolabo with a range of pH 6.0–8.0.

Prior to quantification of PCE, TCE and *cis*-DCE, the target components were extracted 2 min from the headspace using a 100 μm polydimethylsiloxane (PDMS) coated fibre. Quantification was performed on a Fisions 8000 series GC equipped with CP-Sil8 column (25 m × 0.53 mm × 5.0 μm) with helium as carrier gas and a FID detector. The temperature programme started at 50 °C, ramped at 20 °C/min to 140 °C and held at 140 °C for 1.5 min. Injection was splitless (250 °C). VC and ethene were quantified by direct injection of 100 μL (using a glass syringe) on a HP6890 series GC equipped with a CP PoraBond Q column (25 m × 0.53 mm × 10 μm). Temperature was isothermal at 60 °C and detection was done by FID. CO_2 was quantified on a Shimadzu 2010 GC with Thermal Conductivity Detector (TCD) and with helium as carrier gas. Loop injection of 2 mL headspace sample was performed at 120 °C.

2.3. Experimental set-up

Redox titrations: 100 g of wet aquifer material and 250 mL anaerobic tap water were added into 500 mL double-side arm bottles (Fig. 1) inside an anaerobic hood filled with 95% N_2 and 5% H_2. Bottles were closed by Teflon caps with gas tight connected redox electrodes. After removal from the hood, the headspace of the bottles was exchanged in

[3] http://www.mathworks.nl/.
[4] http://www.mathworks.nl/help/curvefit/smooth.html.

10 cycles of vacuuming and refilling with 98% N_2 and 2% CO_2 gas by automated headspace exchanger. Bottles were shaken at 150 rpm in a 25 °C cabinet. After 3 days of stabilisation of the redox potential reading, 1 mL from 18.9 g/L AA stock or 28.1 g/L SL stock solution was added. After day 5, 1.65 mL AA stock or 1 mL SL stock (each time) was also added when the redox potential was stable for at least 2 days (see Figs. S2 and S3). No addition of AA or SL was performed for the blanks. In total 6 additions of AA or SL were carried out in a period of 19 days. The experiment was stopped on day 21, when no further decrease of redox potential was observed after the last addition. Redox titrations with AA, SL and blank were performed in duplicate.

PCE reductive dechlorination after pre-treatments: 200 g of wet aquifer material and 500 mL anaerobic tap water in 1 L double-side arm bottles were used in the pre-treatment step. The preparation procedures were the same as in the redox titration tests. All bottles were prepared at the same time, but the SL treatment was performed after the AA treatment for reasons of availability of the redox potential electrodes. Blanks were monitored throughout the whole duration of the experiment.

While the redox titration tests were aimed at determining the minimum amount of electron donor needed, an initial-surplus was used in the pre-treatment step to ensure suitable redox conditions at the start of microbial reductive dechlorination. Therefore at the end of pre-treatment, in total 1.5 mL from 200 g/L AA stock and 2.5 mL from 200 g/L SL stock were added respectively. After pre-treatment, bottles were transferred to the anaerobic hood and material from each pre-treatment and blank was mixed in 2 L beakers and rinsed with a total of around 3 L anaerobic tap water to remove remaining electron donors. The three steps of the rinsing procedure: (1) settling the slurry for 30 min; (2) pouring out the liquid until water level was close to sediment level; and (3) adding 1 L anaerobic tap water were repeated three times. After rinsing, no AA, lactate and VFA were detected in liquid sample.

The reductive dechlorination experiments were performed in 125 mL serum bottles with 20 g pre-conditioned wet aquifer material and 50 mL liquid. Liquid composition differed according to Table 1. Batches were prepared in the anaerobic hood and closed by viton stoppers. Thereafter, the same headspace exchange procedure as mentioned before was performed for each batch bottle. Afterwards bottles were incubated on a shaker at 150 rpm in a 25 °C incubator.

All batches were spiked with 2.5 mL from 105 mg/L PCE stock leading to approximately 1.6 μmol PCE/batch on day 0. Second spiking with 5 mL from the same PCE stock was performed only in AA4 and SL4 batches. Tests of AA1, AA2, SL1 and SL2 lasted around 87 days, whereas AA3, AA4, SL3 and SL4 lasted around 30 days. Because of memory effects, variance in effective biodegradation cannot simply be summarised using average values. Therefore, results of individual biological PCE reductive dechlorination test instead of average values are given in this paper. Examples of the different tests will be shown below; the rest of results are provided in the supplementary material.

3. Results

3.1. Redox titration

The effect of the stepwise addition of AA or SL on redox potential is shown in the bottom of Fig. 2, where ΔRedox potential was calculated as the difference between the average of the blanks and the average of treatment replicates, using the smoothed redox potential value at the moment just before reductant was added (Figs. S2–S4). At day 8, the redox potential in the AA and SL treatment was respectively 189 and 173 mV lower than that in the blank, after addition of approximately 1.07 mmol/L AA or 1.89 mmol/L SL in total after the second addition. These amounts of electron donor are comparable to approximately 6 mmol electron equivalent (eq.) from both AA and SL per batch (80 g dry mass of aquifer material), based on Eq. (1) and (2).

Adding more electron donor did not lower the redox potential much further. Although small fluctuations were observed, ΔRedox potential in both treatments gradually stabilised between −150 and −200 mV, with in total approximately 3.82 mmol/L AA and 5.93 mmol/L SL added by the end of titration (Fig. 2). The average values for the last redox potential reading of AA treatments, SL treatments and blanks were −423, −469 and −270 mV respectively (Figs. S2–S4).

Besides the redox potential, also Fe^{2+} and SO_4^{2-} were monitored as redox indicators, starting with the first addition of electron donor. In the AA treatment, Fe^{2+} increased from

Table 1
Overview of experimental set-up for the reductive dechlorination batch tests after pre-treatment. All dechlorination batches had 20 g wet aquifer material and a total volume of added liquid of 50 mL.

Pre-treatments[a]			Reductive dechlorination[a]					
Code	Electron donor	# of replicates	Code	PCE (105 mg/L)	SL (225 g/L)	Inoculum	Medium	# of replicates
AA	Ascorbic acid	3	AA1	2.5 mL	–	–	47.5 mL	2
			AA2	2.5 mL	0.5 mL	–	47 mL	3
			AA3	2.5 mL	–	5 mL	42.5 mL	3
			AA4	2.5 mL + 5 mL[b]	0.5 mL	5 mL	42 mL	3
SL	Sodium lactate	3	SL1	2.5 ml	–	–	47.5 mL	3
			SL2	2.5 mL	0.5 mL	–	47 mL	3
			SL3	2.5 mL	–	5 ml	42.5 mL	3
			SL4	2.5 mL + 5 mL[b]	0.5 mL	5 ml	42 mL	3
B	Blank	3	B1	2.5 mL	–	–	47.5 mL	3

[a] Abiotic controls with 0.1 g/L $HgCl_2$ were performed in both parts of experiment.
[b] Second PCE spike on day 8.

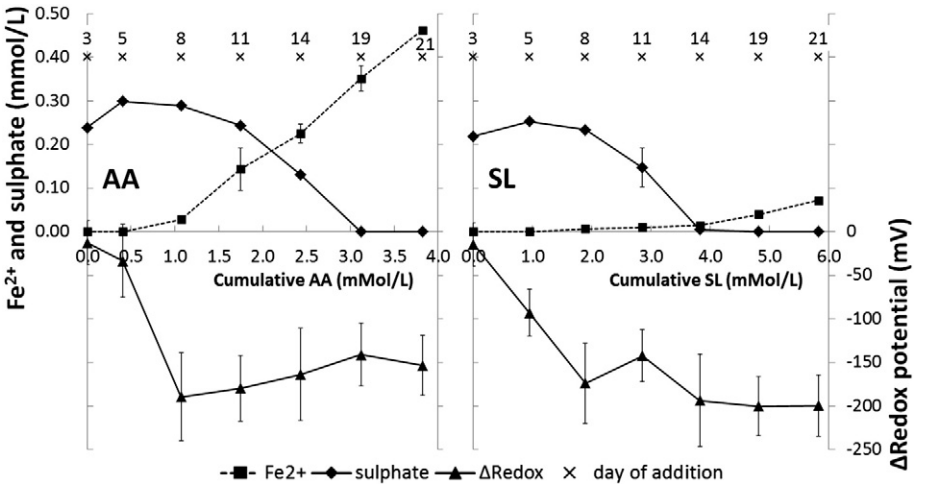

Fig. 2. Fe^{2+} and sulphate evolution (top) and Redox change (bottom) in experimental system with titration of AA (left) and SL (right) respectively, compared to blank. Error bars represent standard errors or duplicates. In some points, errors bars cannot be seen as they are smaller than symbol size. Numbers with crosses indicate the time of measurement and subsequent electron donor additions.

the second addition onwards, at an average rate of 0.03 mmol/L/day, ending up at 0.46 mmol/L (top left of Fig. 2). The increase of Fe^{2+} was much slower in the SL treatment, starting only when SO_4^{2-} had decreased to zero, reaching a concentration of only 0.07 mmol/L by the end of observations (top right of Fig. 2). From a starting concentration of 0.23 mmol/L, SO_4^{2-} was reduced to zero in both treatments (top of Fig. 2). However, the rate of SO_4^{2-} decrease was faster in the SL than in the AA treatment. No Fe^{2+} was detected in the blanks throughout the experiment, but SO_4^{2-} was found to be slowly increasing to an average of 0.33 mmol/L at the end on day 21 (Fig. S5), most probably due to analytical drift.

At the end of experiment no AA or lactate was detected. However, acetate and propionate were present and increased linearly with addition of both AA and SL (Figs. S6 and S7). The final concentrations were 6.49 mmol/L acetate and 0.25 mmol/L propionate in the AA treatment, and 2.37 mmol/L acetate and 3.13 mmol/L propionate in the SL treatment. In addition, CO_2 concentration in the headspace increased in both treatments as well, with the highest rate in the AA treatment (Fig. S8). The developing trends of CO_2 are similar to that of Fe^{2+}. The final amounts of CO_2 in the headspace were 1.19 and 0.26 mmol in the AA and SL treatment respectively, while CO_2 in blanks always remained close to 0.10 mmol (Fig. S8). At the end of redox titration experiment, the carbon mass balance was approximately 77% in the AA treatment and 81% in the SL treatment. In all batches, the pH was neutral throughout the experiment.

These results showed that the aquifer material can be changed from a redox potential at −250 mV, which is regarded at Fe(III) reducing conditions, to a redox potential at −400 mV or lower without reducing all Fe(III). It was shown that both iron and sulphate reduction was initiated. Sulphate reduction led to the conversion of sulphate till below the level of detection. As reductive dechlorination is reported to occur at sulphate reducing conditions it can be expected that at the set redox potential reductive dechlorination may occur.

3.2. PCE reductive dechlorination tests after pre-treatment

Before PCE reductive dechlorination was tested at different conditions, 3.19 mmol/L AA and 8.41 mmol/L SL were added respectively. As explained in the Materials and methods section, the purpose was to condition the aquifer material and set the redox potential to below −400 mV and to reach a comparable condition to redox titration.

During pre-treatment, the observed changes in redox potential, Fe^{2+} and sulphate in the AA and SL treatments were similar to those in the redox titration described above. After the redox potentials stabilised at −450 mV for more than 5 days, the pre-treated aquifer materials were rinsed as described in the Materials and methods section, and PCE reductive dechlorination tests started with different conditions summarised in Table 1.

For the aquifer material that was previously conditioned with AA or SL, in the natural attenuation (NA) tests without stimulation by either additional electron donor or inoculum, a lag phase of at least 30 days was observed before PCE started to degrade. The degradation was incomplete during the experiment and stopped at *cis*-DCE in AA1 and TCE in SL1 (Fig. 3A and B and Fig. S9A and B). PCE degradation did not occur in B1 where aquifer material was not conditioned (Fig. 3C and Fig. S9C).

In the electron donor (ED) stimulation tests, AA2 and SL2 behaved similarly (Fig. 4). In general PCE was degraded with smaller lag phase compared to the NA tests; however, in one of the three AA2 batches no PCE degradation occurred throughout the experiment (Fig. S10A). In AA2, the first appearance of TCE was on day 16, while in SL2, it was observed on day 9. Additionally, *cis*-DCE was detected in one SL2 batch at the last sampling time (Fig. S10B).

In the bioaugmentation tests, complete PCE reductive dechlorination to ethene, with appearance of all intermediate VOCls, was achieved within 30 days (Fig. 5). The first decrease in PCE was already measured after 4 days. In AA3, VC seemed present as the only VOCl compound at day 10

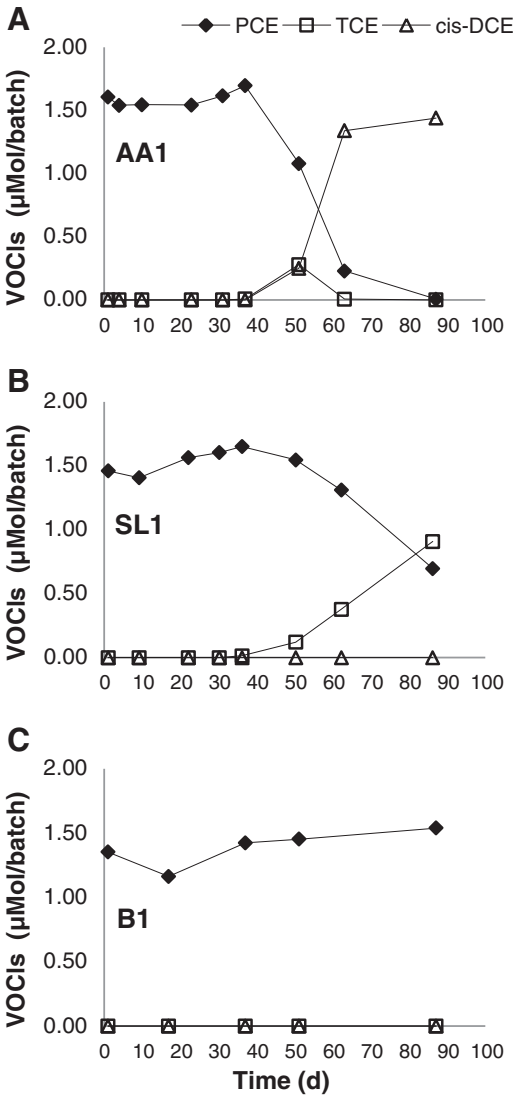

Fig. 3. PCE reductive dechlorination for different experimental conditions (see Table 1) in natural attenuation tests.

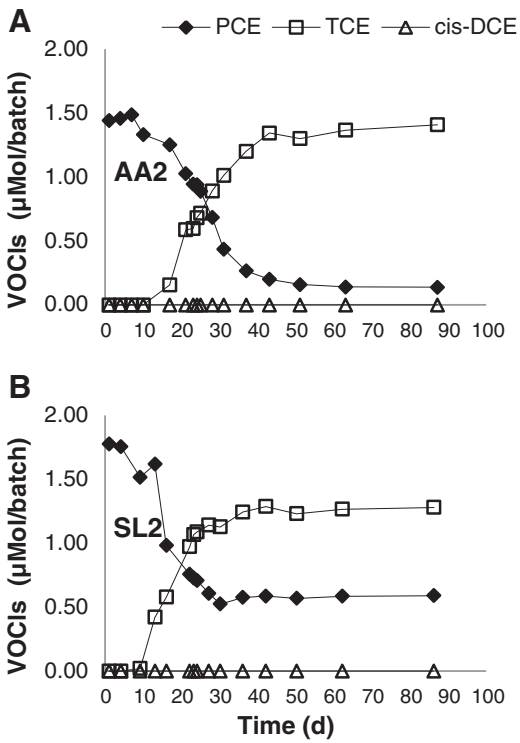

Fig. 4. PCE reductive dechlorination for different experimental conditions (see Table 1) in electron donor stimulation tests.

(Fig. 5A and Fig. S11A). However, ethene was detected after 20 days and the only remaining final product in all batches (Fig. S11).

In the ED + bioaugmentation tests, PCE was degraded within 7 days without a lag phase (Fig. 6). A second PCE spiking was also removed within 5 days and complete conversion to ethene was found in all batches at day 28. In contrast to bioaugmentation tests without additional electron donor, higher accumulation of VC was observed between day 10 and 20 in SL4 than in AA4 (Fig. S12).

The summary of the performance of PCE reductive dechlorination in different scenarios mentioned above is given in Table 2. The VOCl mass balance for the experiments is shown in Table S1. In all abiotic controls, biodegradation of PCE did not occur (Fig. S13).

4. Discussion

4.1. Redox titration

Results from the redox titration revealed that, for the selected PCE contaminated aquifer that was initially under Fe(III) reducing conditions, a minimum addition of 75 µmol electron eq./g dry mass of aquifer material was needed to obtain suitable redox conditions. Additionally, electron donor that provides this amount of electron equivalents seems not specific, as AA and SL showed a similar final redox potential of −450 mV. Interestingly changes in Fe^{2+} and SO_4^{2-} could only be observed after the redox potential had been lowered. A possible explanation for this observation is provided by Christensen et al. (2000) who stated the reduction of ferric aqua ions to ferrous aqua ions is rapid while the reduction of structural Fe(III) to form ferrous aqua ions is a much slower reaction. The observed, initially rapid decrease of redox potential in our study might thus be related to the reduction of a limited amount of aqueous electron acceptors. The slow increase of Fe^{2+} which was observed after the second addition of AA or SL then related to the reduction of structural Fe(III).

Based on Eqs. (1)–(4), it should be noted that the amount of electrons accepted by Fe(III) and SO_4^{2-} is limited compared to the total amount of electron donor added. As normally the contribution of Mn(IV) will be minor compared to Fe(III), and NO_3^- was not present in the experiments, the explanation could be that large part of the electron donor added was consumed in other processes, such as fermentation and

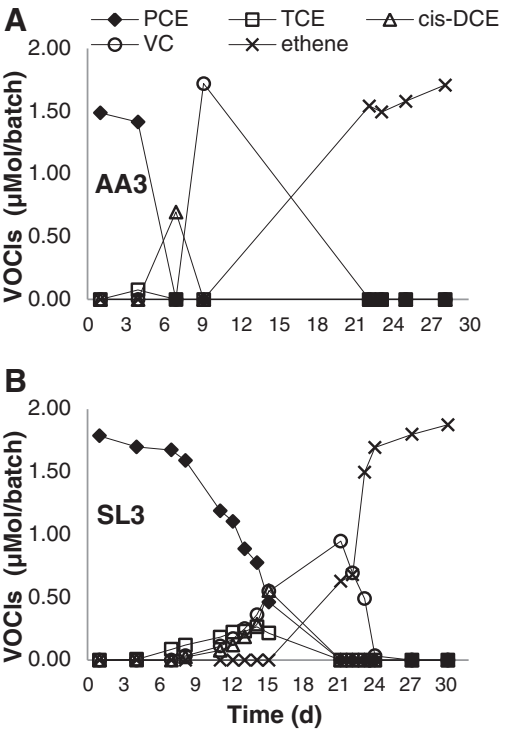

Fig. 5. PCE reductive dechlorination for different experimental conditions (see Table 1) in bioaugmentation tests.

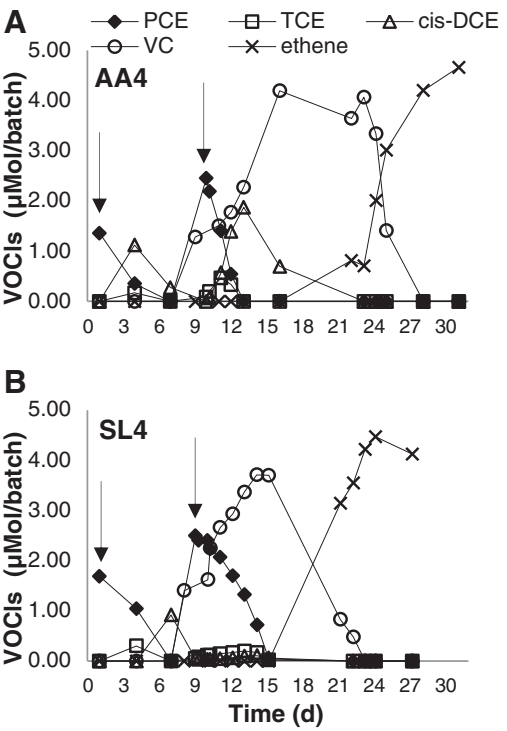

Fig. 6. PCE reductive dechlorination for different experimental conditions (see Table 1) in electron donor + bioaugmentation tests. Arrows indicate two times of PCE spiking.

growth of bacteria. Lactate can be fermented to acetate and propionate by some bacteria via the following reaction (Seeliger et al., 2002):

$$3CH_3CH(OH)COO^- \rightarrow CH_3COO^- + 2CH_3CH_2COO^- + HCO_3^- + H^+ \quad (5)$$

Based on the amount of acetate and propionate measured in the experiment, the fermentation of lactate could be the major process of lactate consumption. Meanwhile, Fe(III) and SO_4^{2-} were reduced by lactate, acetate and propionate. Such fermentation process of AA might be also possible. However, no specific evidence from literature could be found to support this hypothesis. After all, fermentation processes were reported to be important for PCE dechlorination, especially for acetate, in a PCE biodegradation by chitin fermentation (Brennan et al., 2006).

In the experiment, the AA treatment showed a faster rate in Fe^{2+} production, while the SL treatment showed a faster rate in SO_4^{2-} reduction. Probably in the AA treatment most Fe^{2+} chelated with ascorbic acid as ferrous–ascorbate complex (Gorman and Clydesdale, 1983; Plug et al., 1984). This complexation process kept Fe^{2+} in a dissolved phase to be easily detected. In SL treatment, complexation between Fe^{2+} and lactate was probably much less, driving the transformation from SO_4^{2-} to sulphide, then to iron sulphide precipitate faster.

4.2. PCE reductive dechlorination tests after pre-treatment

No reductive dechlorination occurred under fully NA condition where no preconditioning with AA or SL was performed. This was due to the presence of competitive electron acceptors, such as Fe(III), Mn(IV) and sulphate. PCE reductive dechlorination is reported to be possible at redox potential below −200 mV referenced to SHE (ITRC, 2005; Wiedemeier et al., 2007), which was close to the final redox potential, approximately −450 mV referenced to Ag/AgCl electrode, of the pre-treated aquifer material in this study. Therefore in batches that received preconditioning with AA or SL, PCE reductive dechlorination could be stimulated as available Fe(III) and sulphate have been reduced. Besides, acetate as the fermentation product during the pre-treatment could facilitate the growth of "Dehalococcoides" strains (Futagami et al., 2011). Hence, the growth of dechlorinating bacteria in batches received AA or SL before could play an important role, causing the different PCE biodegradation performance between pre-treated and non-pre-treated conditions. Further, stimulations with extra ED after preconditioning showed much earlier and faster reductive dechlorination compared to NA, but dechlorination process was not complete. Reductive dechlorination stopped at TCE probably because of the lack of microorganisms that can perform full reductive dechlorination (Smidt and Vos, 2004).

After bioaugmentation, reductive dechlorination was complete from PCE to ethene, in spite of a few days' lag phase which was probably due to low concentration of electron donor. With extra ED and bioaugmentation, PCE reductive dechlorination occurred directly with complete reductive dechlorination in the end. Evolutions of VOCls were in line with literature (Aulenta et al., 2002; de Bruin et al.,

Table 2
Comparison of the performance on PCE reductive dechlorination in different scenarios.

Feature	Scenarios[a]								
	No pre-treatment	With pre-treatment (AA or SL as indicated)							
		Natural attenuation		Electron donor (ED)		Bioaugmentation (Bio)		ED + Bio	
	B1	AA1	SL1	AA2	SL2	AA3	SL3	AA4	SL4
Reductive dechlorination	−	+		+		+		+	
Lag phase (day)	n.a.	37	30	16	9	<4		0	
End products	PCE	PCE, TCE, *cis*-DCE		PCE, TCE, *cis*-DCE		ethene		ethene	
PCE degradation rate	n.a	Very slow		Slow		Fast		Very fast	

[a] Whether reductive dechlorination occurred or not is indicated by "+" or "−." Not applicable is indicated as n.a.

1992). In addition, the PCE removal rates in all inoculated treatments were comparable to earlier studies (Lee et al., 2001; Suarez and Rifai, 1999). Further, in most cases, ethene as a harmless end product from PCE reductive dechlorination, appeared around 20 days, which was similar to what Kao et al. (2003) showed in their experiments.

Apparently, performance of PCE reductive dechlorination is enhanced in the direction from natural conditions to more stimulated conditions. However, in any scenarios, costs such as time and additional chemicals or bacteria are inevitable for achieving specific purposes.

5. Conclusions and implications

1. PCE reductive dechlorination was stimulated after improving the redox condition of the selected aquifer material by pre-treating with AA or SL.
2. 75 μmol electron equivalents per gram dry mass of aquifer material was the threshold to obtain a redox potential of −450 mV, which is theoretically suitable for PCE reductive dechlorination.
3. The impact of AA and SL on redox potential was not specific, while Fe(III) reduction by AA and SL behaved differently. The results showed faster iron mobilisation by AA. This might induce a risk of iron oxide precipitation, when the mobilised iron, with groundwater extraction or transportation, is exposed to higher redox conditions.
4. Dechlorinating bacteria were needed to achieve complete reductive dechlorination from PCE to ethene, for the selected aquifer material.

The findings of this paper are relevant for improving the cost-effectiveness of the design and operation of in-situ bioremediation. The redox potential of an aquifer can be used as a general indicator to evaluate the potential of PCE reductive dechlorination. The result from our step-wise experiments can be meaningful for dealing with in-situ bioremediation and for applications in large volumes of contaminated aquifer. For achieving specific goals of in-situ bioremediation projects on different VOCls contaminated sites with various environmental conditions, the balance between cost and profit, and potential risks (e.g. bio-chemical well clogging due to bacteria growth and precipitation of metals) should be estimated before the design and operation. When addition of electron donors for improving redox conditions is necessary, the selection of electron donor is also of importance from a cost-effectiveness as well as optimization point of view.

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Appendix A. Supplementary data

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